79. (New) The kit of claim 78, wherein the enzyme is peroxidase or alkaline phosphatase.

REMARKS

Applicants have received and reviewed an Office Action dated November 26, 1996. By way of response, Applicants have canceled claims 1-21 without prejudice or disclaimer of the subject matter therein. Applicants present new claims 22-79. No new matter is presented. Claims 22-79 are pending. Applicants submit the newly presented claims are supported by the specification.

For the reasons given below, Applicants submit the newly presented claims are in condition for allowance and notification to that effect is earnestly solicited.

Petition for Extension of Time

It is noted that a two month petition for extension of time is necessary to provide for timeliness of the response. A request for such an extension is made extending the time for response from February 26, 1997 to April 26, 1997.

Election/Restriction

Applicants acknowledge that the Examiner is continuing examination of the claims of Group I and Species III. Applicants have canceled the claims withdrawn from consideration.

Specification

The Examiner objected to certain informalities in the specification. Amendments to the specification listed

hereinabove correct the typographical errors noted by the Examiner.

Rejection of Claims Under 35 U.S.C. § 112 Second Paragraph

The Examiner rejected claims 1, 6-13 and 16-17 under 35 U.S.C. § 112 second paragraph. The Examiner objected to certain organizational indicia and terms used in the claims. Although this rejection has not been raised for the newly presented claims, it is discussed insofar as it might apply.

The newly presented claims are organized as suggested by the Examiner, do not include the terms and phrases objected to by the Examiner, and address the informalities pointed out by the Examiner. Hence, it is respectfully submitted that this rejection does not apply to the newly presented claims.

Accordingly, it is believed that the newly presented claims fully comply with Section 112 second paragraph, and withdrawal of this rejection is respectfully requested.

Rejections of Claims Under Section 103

The Examiner rejected claims 1, 7, and 17 under 35 U.S.C. § 103(a) as obvious over Widder et al. (EP 016,552) in view of Connelly et al. (U.S. Patent No. 5,422,277), Forrest et al. (U.S. Patent No. 4,659,678), and Pilling et al. (Journal of Immunological Methods 1989). Although this rejection has not been raised for the newly presented claims, it is discussed insofar as it might apply. Applicants respectfully traverse the rejection.

The Widder et al. reference cited by the Examiner uses Protein A to bind antibodies to magnetic particles. The Protein A binding is required to properly orient the antibodies (Widder

et al., Col. 3, lines 31-43). Furthermore, Protein A binds to the Fc-portion of an antibody. This binding, however, has too little specificity and impossible to use in the present invention, since the requirements for specific binding is of crucial importance. The requirement for specificity in the present invention is so high that no person with skill in the art would think of using the teaching of the unspecific protein A/Ig system to invent a method such as the present one. Applicants' invention is distinct from the disclosure of Widder et al. at least in the feature of attaching an antibody directly to the magnetic particle. Applicants' invention does not require precoating the paramagnetic particle or bead with Protein A. Hence, Widder et al. neither teaches nor suggests the claimed invention.

As explained hereinbelow, the secondary references do not remedy the shortcomings of the Widder et al. reference.

The Examiner seems to emphasize the use of fixatives to pretreat the samples, as disclosed by Connelly et al. As a matter of fact the use of fixatives is not a feature of the presently claimed invention. Pretreating with fixatives is only necessary if one wants to study the bead-identified cells further by the use of antibodies which bind to intracellular receptors, and is not used nor warranted for selected and direct identification of bead-rosetting target cells using antibodies directed to extracellular membrane antigen determinants.

Regarding the Examiners comments on the biotin-avidin system of Forrest et al., we have the following comments. The avidin/biotin system could be used as part of one of many different means for additional characterization of the target cells. This is, however, not a necessary part of the presently

claimed invention because the target cells are visualized by the bound beads. By looking into the microscope one can identify the malignant cells since they have beads attached to them. Furthermore, Protein A binds to the Fc-portion of an antibody. this binding is, however, not sufficiently specific and impossible to use in the present invention, since the requirements for specific binding is of crucial importance. The requirement for specificity in the present invention is so high that no person with skill in the art would think of using the teaching of the unspecific protein A/Ig system to invent a method such as the present one.

Regarding reaction temperature and time, disclosed by Pilling et al., Applicants respectfully submit that a person skilled in the art would not optimize such a reaction to the parameters of the present invention. Pilling et al. regards optimization of negative immunoselection which is a different method than the positive selection of the present invention. Furthermore, Pilling et al. found that 90 minutes incubation period was optimal. Pilling et al. used CD4+ T cells which mostly are considerably smaller than, for example, the tumor cells selected from the solid cancers exemplified in the present study. To magnetically isolate larger cells requires stronger binding which normally makes necessary longer incubation period and higher incubation temperatures. It was therefore an unexpected result that adequate binding in the present method was obtained within 30 minutes at temperatures of 4°C. This would not have been obvious for a person skilled in the art based on the teaching of Pilling et al. It should be emphasized that there is a qualitative major difference between normal

hematopoetic cells (CD4+ T-cells) and normal and malignant cells from other tissues.

Accordingly, it is respectfully submitted that the claimed invention is neither taught nor suggested by the references cited by the Examiner, either alone or in combination, and withdrawal of this rejection is respectfully requested.

The Examiner rejected claims 6, and 8-13 under 35 U.S.C. § 103(a) as obvious over Widder et al. in view of Connelly et al., Forrest et al., and Pilling et al. and further in view of Kemmer et al. (Journal of Immunological Methods 1992), and Holmes et al. (WO 91/09938). Although this rejection has not been raised for the newly presented claims, it is discussed insofar as it may apply. Applicants respectfully traverse this rejection.

The Widder et al., Connelly et al., Forrest et al., and Pilling et al. references are discussed hereinabove. The deficiencies of these cited references are not remedied by the Kemmer et al. and/or the Holmes et al. references.

Applicants respectfully bring to the Examiner's attention that the present method relates to positive selection of target cells and that the beads remain attached to the target cells, with a very high specificity, such that the beads (bead rosettes) can be used for identification of the target cells. Applicants reiterate that one feature of the method, namely that it is a method wherein the antibody-bead complex is used to select, with very high specificity, binding to target cells such that these can be identified by the bead-rosettes attached to target cells.

Kemmer et al. use the beads for enriching the cell population prepared from a solid tumor containing mainly tumor cells, and they use the bead/cell-complex to assess the effect of

the enrichment. However, only 96% of the bead-rosetting cells with the specific antibody proved to be tumor cells and 5% of the cells attached to the beads coated only with an irrelevant antibody directed against murine antibodies with tumor cells. Moreover, of the 34% cells that bound beads coated with an antibody recognizing the human leukocyte common antigen (Dako-LC), as much as 35% turned out to be tumor cells. This is a good example of the lack of specificity in the method of Kemmer et al., since Dako-LC antigens are not expressed on the tumor cells. These data demonstrate a highly nonspecific method which can not be used to specifically diagnose and detect target cells in a mixed cell population, and that the results teach the present invention is impossible.

Holmes et al. regard a combination of positive and negative selection wherein both procedures can be performed in opposite succession and the beads are detached and removed from the cells. The method is performed with hematopoietic cells, and there is no required specificity since this specificity is not required.

The present invention is not obvious in light of Holmes et al. and Kemmer et al. due to that both methods are entirely different from the present method and not sufficiently specific according to the requirements inherent in the present method. For example, to detect tumor cells in blood and bone marrow is profoundly more difficult. If there are a couple of malignant cells in a population of one hundred thousand or millions of normal cells, the present method must be able and is able to pick them out. Therefore the present method is not obvious for a person skilled in the art based on the teaching of Holmes et al. and Kemmer et al.

Accordingly, based on the foregoing differences, it is respectfully submitted that the claimed methods are neither nor suggested by the references cited by the Examiner and withdrawal of this rejection is respectfully requested.

The Examiner rejected claim 16 under 35 U.S.C. § 103 as obvious over Widder et al. in view of Connelly et al., Forrest et al., and Pilling et al., and further in view of Afseth et al. (WO 91/15766). The Widder et al., Connelly et al., Forrest et al., and Pilling et al. references have been discussed hereinabove. The Afseth et al. reference does not remedy the deficiencies of the previously discussed references.

Afseth et al. teach a method for cleaving antigen/anti-antigen, hapten/anti-hapten bindings. Hence, they use antibodies or antibody fragments to remove the beads from the cells. They also claim a kit for use in their method, the kit containing solid support bound to an antibody or anti-hapten, and antibody fragment which binds to the antibody or anti-hapten which competitively binds to the antigen on the cell surface. The kit of Afseth et al. is suited to their method only. The kit of the present invention is of course designed according to the present method and as such it is novel and non-obvious, since it is impossible that the kit could be predicted from any other publication. Hence, Afseth neither teaches nor suggests the presently claimed invention.

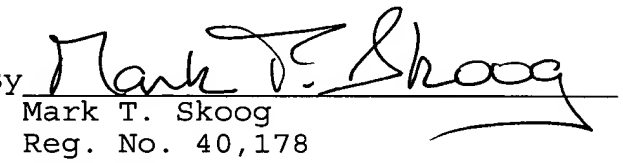
Accordingly, based on the foregoing differences, it is respectfully submitted that the cited references, either alone or in combination, neither teach nor suggest the claimed method and withdrawal of this rejection is respectfully requested.

Summary

In summary, Applicants submit that each of claims 22-79 are in condition for allowance.

Respectfully submitted,

MERCHANT, GOULD, SMITH, EDELL,
WELTER & SCHMIDT, P.A.
3100 Norwest Center
90 South Seventh Street
Minneapolis, MN 55402
(612) 332-5300

By 
Mark T. Skoog
Reg. No. 40,178
MTS:jae